

sample introduction port of a cartridge and, preferably, seals a closure on the sample introduction port. The cartridge is inserted into the cartridge reader. Preferably, the cartridge will include features that ensure the cartridge is inserted in the proper orientation; e.g., by incorporating identifying marks to show which direction it should be placed on the tray and/or mechanical features that guide the user to place it in the correct orientation. After the user has successfully prepared and inserted the cartridge, reading/processing of the cartridge is performed by the cartridge reader upon receiving an indication from the user that the read cycle should commence (alternatively, the reader may automatically begin operation upon confirming that a properly prepared cartridge has been properly inserted into the cartridge reader). The subsequent reading of the cartridge is preferably automated; e.g., the cartridge reader's electronic control system (computerized control system or the like) automatically processes and reads the cartridge.

[0269] The automated sequence of operations to be performed by the cartridge reader will now be described. Preferably the cartridge includes machine readable indicia, e.g., barcode, that is detected and processed by the cartridge reader. For example, processing of the machine readable indicia may allow the cartridge reader to verify that a valid, readable barcode has been detected and thereafter determine the operational parameters for the present read cycle; i.e., determine the set of assays/tests to be performed, extract any relevant instrument configuration parameters and verify the expiration date. In certain preferred embodiments, the cartridge reader may prompt the user for any data that it requires; e.g., operator ID, sample or patient ID, and the like. Additionally, if the cartridge is capable of running a panel of test, the user may be able to select which test(s) within the panel should want be performed.

[0270] Preferably, the reader has a cartridge handling sub-system that mechanically engages the cartridges and moves/aligns it into position. Preferably, this process includes positioning the cartridge within a light-tight enclosure. The reader also makes the appropriate fluidic and/or electronic connections to the cartridge and, optionally, breaks or pierces any reagent modules (e.g., reagent ampoules) present in cartridge reagent chambers. As discussed above, in one preferred embodiment, the cartridge handler's motion would be physically coupled to the fluidic and electronic handlers (and, optionally, the reagent module release mechanism) such that upon positioning the cartridge within the light tight enclosure the electrical contacts and the fluidics manifold engage the cartridge at their respective engagement points (and, optionally, the reagent module release mechanisms releases reagent from any reagent modules). Next, where required or preferred, the electronic control system begins operating a heater in order to bring the cartridge to the appropriate predetermined temperature and maintain the cartridge at such target temperature. In certain preferred embodiments temperature regulation may be controlled by a microprocessor employing a proportional derivative control to control a heater that will maintain the target temperature; preferably a suitable algorithm is employed.

[0271] Once the cartridge has been maintained at the target temperature for a predetermined amount of time, the fluid handler may begin processing the cartridge for reading; i.e., assemble the assay. Reference to **FIG. 26** will be made

to illustrate the intermediary states of the cartridge reader and the position of fluid within the fluid network of cartridge **2500** during a 2-step assay format. As presented in **FIG. 26**, the starting state of the cartridge **2500** (panel **2601**) is illustrated and depicts the location of the constituent fluids within the fluidic network. Assay assembly preferably consists of metering specific volumes of sample fluid, reconstituting dried reagents in the sample fluid and incubating the sample fluid in the detection chambers. Predetermined valves are opened in a prescribed sequence in accordance with the desired fluid flow paths to be assumed by the constituent fluids.

[0272] According to the present embodiment in which two read chambers are present and will be utilized for testing the sample, two equal lengths of sample fluid (i.e., slugs) will be drawn; the length of the sample slugs is determined by the volume of the read chambers. The sample slugs are delimited from one another by introducing a slug of air between the two sample slugs. Accordingly, sample chamber vent valve **2412** and a waste chamber vent valve **2442A** are opened and the pump vent is closed. The pump is subsequently activated to aspirate/draw the sample from sample chamber **2510** (preferably, overcoming a capillary break provided by a Z-transition that is used to prevent leakage of the sample from the sample chamber) into sample conduit branch **2515A**. In this and other pumping steps, a pressure sensor (not shown), preferably, detects the pressure created by the operation and provides confirmation that the pump is aspirating/dispersing fluid properly. When fluid is detected at sensor **3** (see **FIG. 26, 2602**), the pump vent valve is opened and the pump motor is deactivated. The sample chamber vent valve **2412** and waste chamber vent valve **2442A** are then closed. Similarly, sample is drawn into sample conduit branch **2515B** by operating the pump with sample chamber vent valve **2412** and waste chamber B vent valve **2442B** open (see **FIG. 26, panel 2603**). Defined slugs of sample fluid are drawn into the sample conduit branches by operating the pump with air vent valve **2422** open as well as the waste chamber A and B vent valves **2442A-B** (see **FIG. 26, panel 2604**). In this and subsequent steps, two slugs may be moved simultaneously through sample conduit branches **2515A** and B by holding both waste chamber vent valves open or sequentially through the branches by opening one at a time.

[0273] The sample conduit branches, preferably, comprise dry reagent pills (preferably containing one or reagents selected from blocking agents, pH buffers, salts, labeled binding reagents, and the like). One or more of the conduit branches may also comprise spiked analyte for spike recovery controls. In order to reconstitute the dried reagent, the two sample fluid slugs are moved back and forth across the pill zone a predetermined number of times by opening air vent valve **2422** and waste chamber vent valves **2442A** and/or B and operating the pump to alternate between applying positive and negative pressure to the waste chamber vents (**FIG. 26, panels 2605-2606**). The two sample fluid slugs may be moved back and forth simultaneously or mixing of the two slugs may be accomplished in series. The number of repetitions that the sample fluid is cycled across the pill zone may be dependent upon a number of factors, including but not limited to, size/volume of reagent dried reagent pill, composition of reagent pill, drying method employed at the time of reagent deposition/pill formation, and the like. In accordance with preferred embodiments, the